

**Figure 1. N-linked oligosaccharides for glycosylation**

Peptide sequence (SEQ ID NO:5):

```
MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNPEEGDLN
PPPEAKQVPV SYYDSTYLST DNEKDNYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
STIDTELKVI DTNCINVIQP DGSYRSEELN LVIIGPSADI IQFECKSFGH EVLNLTRNGY
GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GK FATDPAVT LAHELIIHAGH RLYGIAINPN
RVFKVNTNAY YEMSGLEVSF EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIAS TLNKA
KSI VGT TASL QYMKNVFKEK YLLSEDTS GK FSVDK LKFDK LYKMLTEIYT EDNFVKFFKV
LNRKTYLNFD KAVFKINIVP KVNYYIYDGF NLRNTNLAAN FNGQNT EINN MNFTKLKNFT
GLFEFYKLLC VRGIITSKTK SLDKGYNKAL NDLCIKVNNW DLFFSP SEDN FTNDL NKGEE
ITS D TNIEAA EENISLDLIQ QYYLTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG
KKYELDKYTM FHYLRAQEFE HGKSRIALT N SVNEALLNPS RVYTF FSSDY VKKVNKATEA
AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMLYKD DFGALIFSG
AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTI DNALS KRNEKWDEVY KYIVTNWLAK
VNTQIDLIRK KMKEALENQA EATKAIINYQ YNQYTEEEKN NINFNIDDL S SKLNESINKA
MININKFLNQ CSVSYLMNSM IPYGVK RLED FDASLKDALL KYIYDNRGTL IGQVDRLKDK
VNNTLSTDIP FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSR YASKINI
GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWIRIP KYFNSISLNN
EYTIINC MEN NSGWK VSLNY GEIIWTLQDT QEIKQRV VFK YSQMINISDY INRWIFVTIT
NNRLNNSKIY INGR LIDQKP ISNLGNIHAS NNIMFKLDGC RDTHRYIWIK YFNLF DKELN
EKEIKDLYDN QSNSGILKDF WGDYLQYDKP YYMLNLYDPN KYVDVNNVGI RGYMYLKGPR
GSVMTTNIYL NSSLYRGTKF I IKKYASGNK DNIVRNNDRV YINVVVKNKE YRLATNASQA
GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI GFHQFN NIAK
LVASNWYNRQ IERSSRTLGC SWEFIPVDDG WGERPL
```

**Peptides containing the motif 'N-X-S/T/C (X not P)' (underlined):**

| position  | Peptide                                              | SEQ ID NO: |
|-----------|------------------------------------------------------|------------|
| 167-177   | SFGHEVL <u>NL</u> TR                                 | 6          |
| 382-393   | VNYTIYDGFNLR                                         | 7          |
| 394-415   | NTNLAANFNGQNT EINN <u>MN</u> FK                      | 8          |
| 418-427   | <u>NFT</u> GLFEFYK                                   | 9          |
| 457-477   | VNNWDLFFSP SEDN <u>NFT</u> NDLN K                    | 10         |
| 478-536   | GEEITS D TNIEAAEENISLD LIQQYYLTFNFDNEP <u>ENIS</u> I | 11         |
| 773-779   | ENLSSDIIGQLELMPNIER                                  | 12         |
| 773-779   | LNESINK                                              | 12         |
| 787-806   | FLNQCSVS YLMNSMIPYGVK                                | 13         |
| 841-855   | V <u>NN</u> TLSTDIPFQLSK                             | 14         |
| 872-882   | NI <u>INT</u> SILNLR                                 | 15         |
| 930-948   | NAIVYNSMYEN <u>FST</u> SFWIR                         | 16         |
| 952-975   | YFNSISLNN EYTIINCME <u>NN</u> SGWK                   | 17         |
| 1001-1013 | YSQMINISDYINR                                        | 18         |
| 1024-1028 | L <u>NN</u> SK                                       | 19         |
| 1086-1098 | DLYDNQSN SGILK                                       | 20         |
| 1141-1156 | GSVMTTNIYLNSSLYR                                     | 21         |
| 1193-1204 | LATNASQAGVEK                                         | 22         |
| 1205-1224 | ILSALEIPDVGNLSQVVVMK                                 | 23         |

**Figure 1 Continue.**

**Peptide sequence (SEQ ID NO: 24):**

KTKSLDKGYN KALNDLCIKV NNWDLFFSPS EDNFTNDLNK GEEITSDTNI EAAEENISLD  
 LIQQYYLTFN FDNEPENISI ENLSSDIIGQ LELMPNIERF PNGKKYELDK YTMFHYLRAQ  
 EFEHGKSRIA LTNSVNEALL NPSRVYTFFS SDYVKKVNKA TEAAMFLGWV EQLVYDFTDE  
 TSEVSTTDKI ADITIIIPYI GPALNIGNML YKDDFVGALI FSGAVILLEF IPEIAIPVLG  
 TFALVSYIAN KVLTVQTIDN ALSKRNEKWD EVYKYIVTNW LAKVNTQIDL IRKKMKEALE  
 NQAEATKAI NYQYNQYTEE EKNNINFNID DLSSKLNESI NKAMININKF LNQCSVSYLM  
 NSMIPYGVKR LEDFDASLKD ALLKYIYDNR GTLIGQVDRL KDKVNNTLST DIPFQLSKYV  
 DNQRLSTFT EYIKNIINTS ILNLRYESNH LIDLSRYASK INIGSKVNFD PIDKNQIQLF  
 NLESSKIEVI LKNAIVYNSM YENFSTSFWI RIPKYFNSIS LNNEYTIINC MENNSGWKVS  
 LNYGEIIWTL QDTQEIKQRV VFKYSQMINI SDYINRWIFV TITNNRLNNS KIYINGRLID  
 QKPISNLGNI HASNNIMFKL DGCRDTHRYI WIKYFNLFDK ELNEKEIKDL YDNQNSNGIL  
 KDFWGDYLQY DKPYMLNLY DPNKYVDVNN VGIRGYMYLK GPRGSVMTTN IYLNSSLYRG  
 TKFIIKKYAS GNKDNIVRNN DRVYINVVVK NKEYRLATNA SQAGVEKILS ALEIPDVGNL  
 SQVVMKSKN DQGITNKCKM NLQDNNGNDI GFIFGHQFNN IAKLVASNWY NRQIERSSRT  
 LGCSWEFIPV DDGWERPL

**Peptides containing the motif 'N-X-S/T/C (X not P)':**

| position | peptide                                                          | SEQ ID NO: |
|----------|------------------------------------------------------------------|------------|
| 20-40    | VNNWDLFFSPSEDNFTNDLN K                                           | 25         |
| 41-99    | GEEITSDTNIEAAEENISLD LIQQYYLTFNFDNEPENISI<br>ENLSSDIIGQLELMPNIER | 26         |
| 336-342  | LNESINK                                                          | 27         |
| 350-369  | FLNQCSVSYLMNSMIPYGVK                                             | 28         |
| 404-418  | VNNTLSTDIPFQLSK                                                  | 29         |
| 435-445  | NIINTSILNLR                                                      | 30         |
| 493-511  | NAIVYNSMYENFSTSFWIR                                              | 31         |
| 515-538  | YFNSISLNNEYTIINCMENN SGWK                                        | 32         |
| 564-576  | YSQMINISDYINR                                                    | 33         |
| 587-591  | LNNSK                                                            | 34         |
| 649-661  | DLYDNQNSNGILK                                                    | 35         |
| 704-719  | GSVMTTNIYLNSSLYR                                                 | 36         |
| 756-767  | LATNASQAGVEK                                                     | 37         |
| 768-787  | ILSALEIPDVGNLSQVVMK                                              | 38         |

**Figure 2.**

**Peptide sequence (SEQ ID NO: 39):**

```

MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT FTNPEEGDLN
PPPEAKQVPV SYDSTYLST DNEKDNYLKG VTKLFERIYS TDLGRMLLTS IVRGIPFWGG
STIDTELKVI DTNCINVIQP DGSYRSEELN LVIIGPSADI IQFECKSFGH EVLNLTRNGY
GSTQYIRFSP DFTFGFEESL EVDTNPLLGA GKFDATPAVT LAHELIIHAGH RLYGIAINPN
RVFKVNTNAY YEMSGLEVSF EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
KSIVGTTASL QYMKNVFKEK YLLSEDTSKG FSVDKLKFDK LYKMLTEIYT EDNFVKFFKV
LNRKTYLNFD KAVFKINIVP KVNYYTIYDGF NLRNTNLAAN FNGQNTTEINN MNFTKLKNFT
GLFEFYKLLC VRGIITSKTK SLDKGYNKAL NDLCIKVNNW DLFFSPSEDN FTNDLNKGEE
ITSDTNIEAA EENISLDLIQ QYYLTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG
KKYELDKYTM FHYLRAQEFH HGKSRIALTN SVNEALLNPS RVTTFSSDY VKKVNKATEA
AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMPLYKD DFVGALIFSG
AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTIIDNALS KRNEKWDEVY KYIVTNWLAK
VNTQIDLIRK KMKEALENQA EATKAIINYQ YNQYTEEEKN NINFNIDDL SSKNESINKA
MININKFLNQ CSVSYLMNSM IPYGVKRLD FDASLKDALL KYIYDNRGTL IGQVDRKDK
VNNTLSTDIP FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSRYSKINI
GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWRIP KYFNSISLNN
EYTIINCMEN NSGWKVSLEY GEIWTLDQT QEIKQRVVFK YSQMINISDY INRWIFVTIT
NNRLNNSKIY INGRLLDQKP ISNLGNIHAS NNIMFKLDGC RDTHRYIWIK YFNLFDKELN
EKEIKDLYDN QSNNGILKDF WGDYLDYDKP YYMLNLYDPN KYVDVNNVGI RGYMYLKGPR
GSVMTTNIYL NSSLYRGTKF IIKKYASGNK DNIVRNNDRV YINVVVKNKE YRLATNASQA
GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI GFHQFNNAK
LVASNWNRYQ IERSSRTLGC SWEFIPVDDG WGERPL

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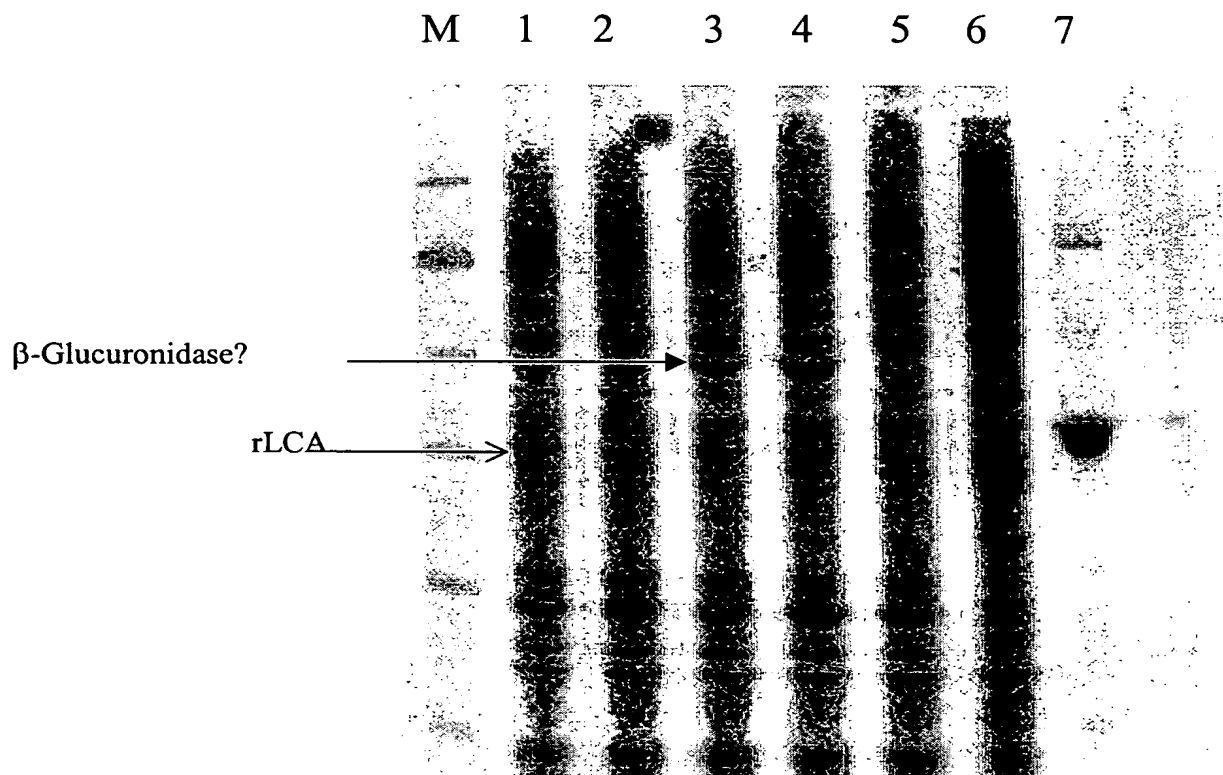
**Peptides containing S or T (underlined):**

| position | peptide                                 | SEQ ID NO: |
|----------|-----------------------------------------|------------|
| 49-66    | DIF <u>TNPEEGDLNPPPEAK</u>              | 40         |
| 67-84    | QVPV <u>SYYDSTYLSTDNEK</u>              | 41         |
| 90-93    | G <u>VT</u> K                           | 42         |
| 98-105   | IY <u>STD</u> LGR                       | 43         |
| 106-113  | ML <u>TS</u> IVR                        | 44         |
| 114-128  | GIPFWGG <u>STIDTELK</u>                 | 45         |
| 129-145  | VIDTNCINVIQPDGSYR                       | 46         |
| 146-166  | <u>SEELNLVIIGPSADIIQFEC</u> K           | 47         |
| 167-177  | <u>SFGHEVLNLTR</u>                      | 48         |
| 178-187  | NGY <u>GSTQYIR</u>                      | 49         |
| 188-212  | FSPDFTFGFEES <u>LEVD</u> TNPL LGAGK     | 50         |
| 213-231  | FATDPAVTLAHELIIHAGHR                    | 51         |
| 245-264  | VNTNAYYEM <u>S</u> GLEVS <u>F</u> EELR  | 52         |
| 265-272  | <u>T</u> FGGHDAK                        | 53         |
| 273-283  | FIDSLQENEFR                             | 54         |
| 292-299  | DIAS <u>T</u> LNK                       | 55         |
| 302-314  | <u>SIVGTTASLQYMK</u>                    | 56         |
| 321-330  | YLL <u>S</u> EDTSKG                     | 57         |
| 331-335  | <u>F</u> SVDK                           | 58         |
| 344-356  | ML <u>TEIYT</u> EDNFVK                  | 59         |
| 365-371  | <u>TY</u> LNFDK                         | 70         |
| 382-393  | VNYTIIYDGFNLR                           | 71         |
| 394-415  | <u>N</u> TNLAANFNGQNTTEINNMNF <u>IK</u> | 72         |
| 418-427  | NFTGLFEFYK                              | 73         |
| 433-438  | GI <u>TS</u> K                          | 74         |
| 439-440  | <u>T</u> K                              | 75         |
| 441-444  | <u>S</u> LDK                            | 76         |

**Figure 2**  
**Continue**

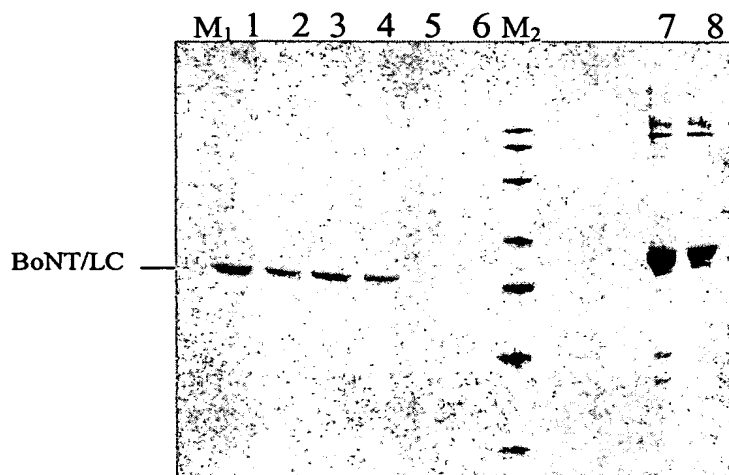
|           |                                                                   |     |
|-----------|-------------------------------------------------------------------|-----|
| 457-477   | VNNWDLFFSPSEDNFTNDLN K                                            | 77  |
| 478-536   | GEEITSDTNIEAAEENISLD LIQQYYLT FNFDNEPENISI<br>ENLSSDIIGQLELMPNIER | 78  |
| 548-555   | YTMFHYLR                                                          | 79  |
| 564-565   | SR                                                                | 80  |
| 566-581   | IALTNSVNEALLNPSR                                                  | 81  |
| 582-592   | VYTFFSDDYVK                                                       | 82  |
| 597-626   | ATEAAMFLGWVEQLVYDFTD ETSEVSTTDK                                   | 83  |
| 627-649   | IADITIIPYIGPALNIGNM LYK                                           | 84  |
| 650-688   | DDFVGALIFSGAVILLEFIP EIAIPVLGTFALVSYIANK                          | 85  |
| 689-701   | VLTVQTIDNALS K                                                    | 86  |
| 712-720   | YIVTNWLAK                                                         | 87  |
| 721-729   | VNTQIDLR                                                          | 88  |
| 734-744   | EALNQAEATK                                                        | 89  |
| 745-759   | AHNYQYNQYTEEEK                                                    | 90  |
| 760-772   | NNINFNIDDLSSK                                                     | 91  |
| 773-779   | LNESINK                                                           | 92  |
| 787-806   | FLNQCSVSYLMNSMIPYGVK                                              | 93  |
| 808-816   | LEDFDA SK                                                         | 94  |
| 828-836   | GTLIGQVDR                                                         | 95  |
| 841-855   | VNNTLSTDIPQLSK                                                    | 96  |
| 862-871   | LLSTFTEYIK                                                        | 97  |
| 872-882   | NIINTSILNLR                                                       | 98  |
| 883-893   | YESNHLIDL SR                                                      | 99  |
| 894-897   | YASK                                                              | 100 |
| 898-903   | INIGSK                                                            | 101 |
| 912-923   | NQIQLFNLESSK                                                      | 102 |
| 930-948   | NAIVYNSMYENFSTSFWR                                                | 103 |
| 952-975   | YFNSISLNNEYTIINCMENN SGWK                                         | 104 |
| 976-994   | VSLNYGEIHWTLQDTQEIK                                               | 105 |
| 1001-1013 | YSQMINISDYINR                                                     | 106 |
| 1014-1023 | WIFVTITNNR                                                        | 107 |
| 1024-1028 | LNN SK                                                            | 108 |
| 1035-1056 | LIDQKPI SNLGNIHASNNIM FK                                          | 109 |
| 1062-1065 | DTHR                                                              | 110 |
| 1086-1098 | DLYDNQSN SGILK                                                    | 111 |
| 1141-1156 | GSVMTTNIYLNSSL YR                                                 | 112 |
| 1157-1159 | GTK                                                               | 113 |
| 1165-1170 | YASGNK                                                            | 114 |
| 1193-1204 | LATNASQAGVEK                                                      | 115 |
| 1205-1224 | ILSALEIPDVGNLSQVVVMK                                              | 116 |
| 1225-1226 | SK                                                                | 117 |
| 1227-1234 | NDQGITNK                                                          | 118 |
| 1261-1269 | LVASN WYNR                                                        | 119 |
| 1274-1276 | SSR                                                               | 120 |
| 1277-1296 | TLGCSWEFIPVDDGWGERPL                                              | 121 |

Figure 3



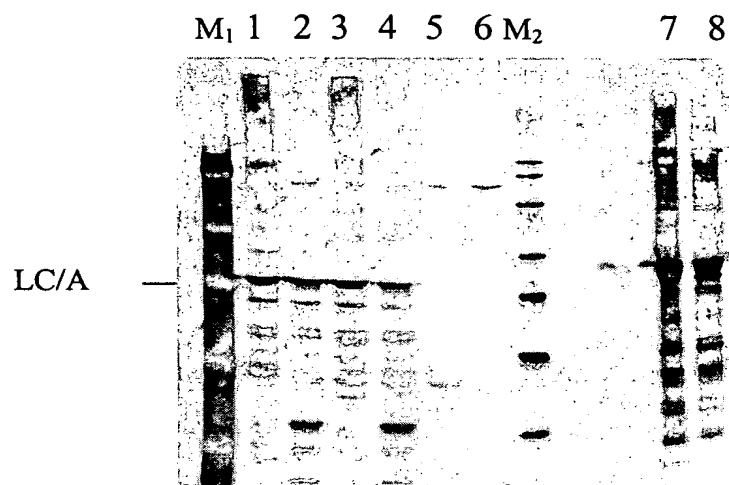
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1. pBAC-1/LCA, H227Y  
2. pBAC-1/LCA  
3. pBACgus-1/LCA, H227Y  
4. pBACgus-1/LCA  
5. AcNPV, negative control  
6. Sf9 insect cells only  
7. E.coli expressed LCA

Figure 4



WB: Anti-His mAb

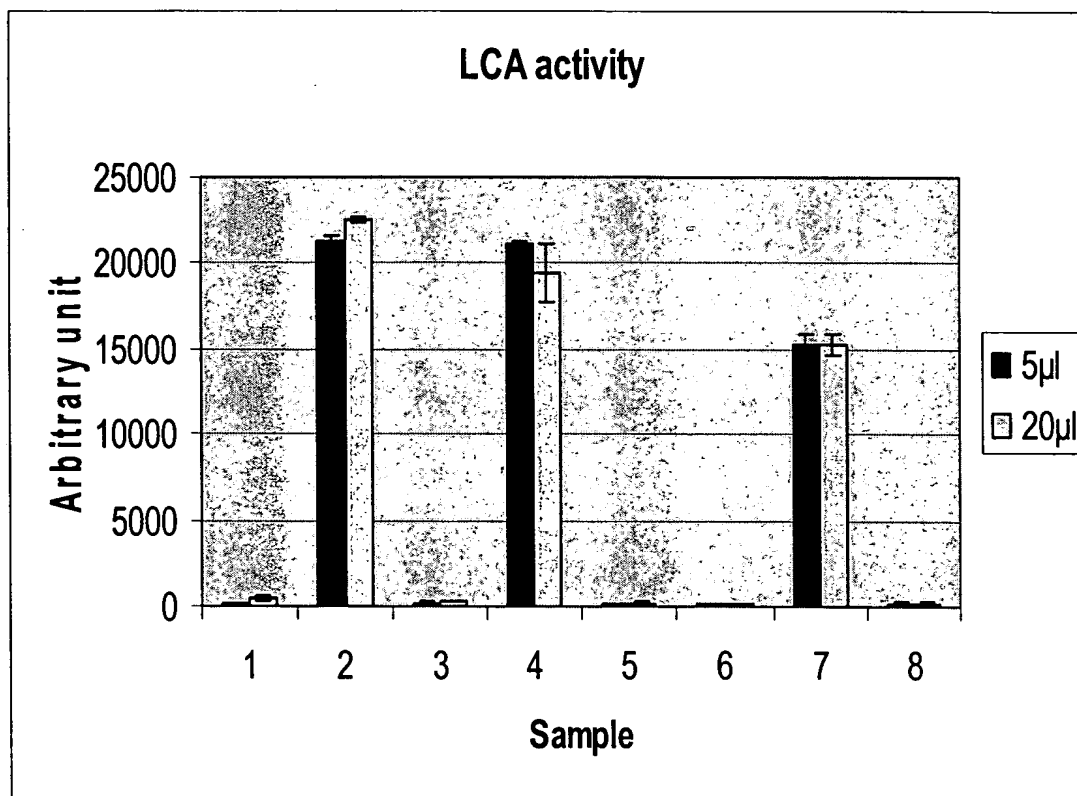
1. pBAC-1/LCA, H227Y
  2. pBAC-1/LCA
  3. pBACgus-1/LCA, H227Y
  4. pBACgus-1/LCA
  5. AcNPV, negative control
  6. Sf9 insect cells only
  - 7, 8, E.coli expressed LCA
- M<sub>1</sub>, SeeBlue Plus2



WB: Anti-LCA pAb

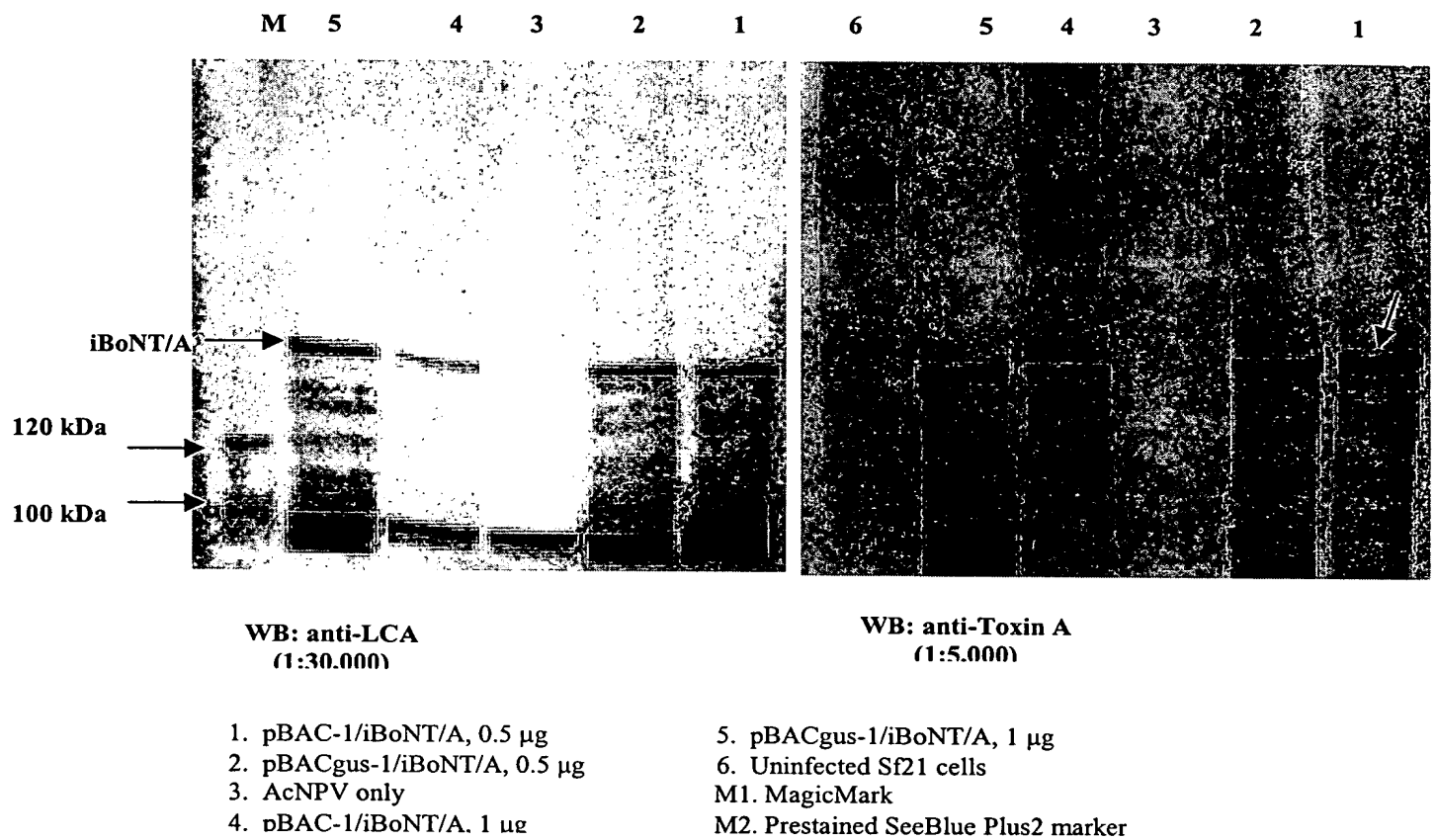
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Figure 5

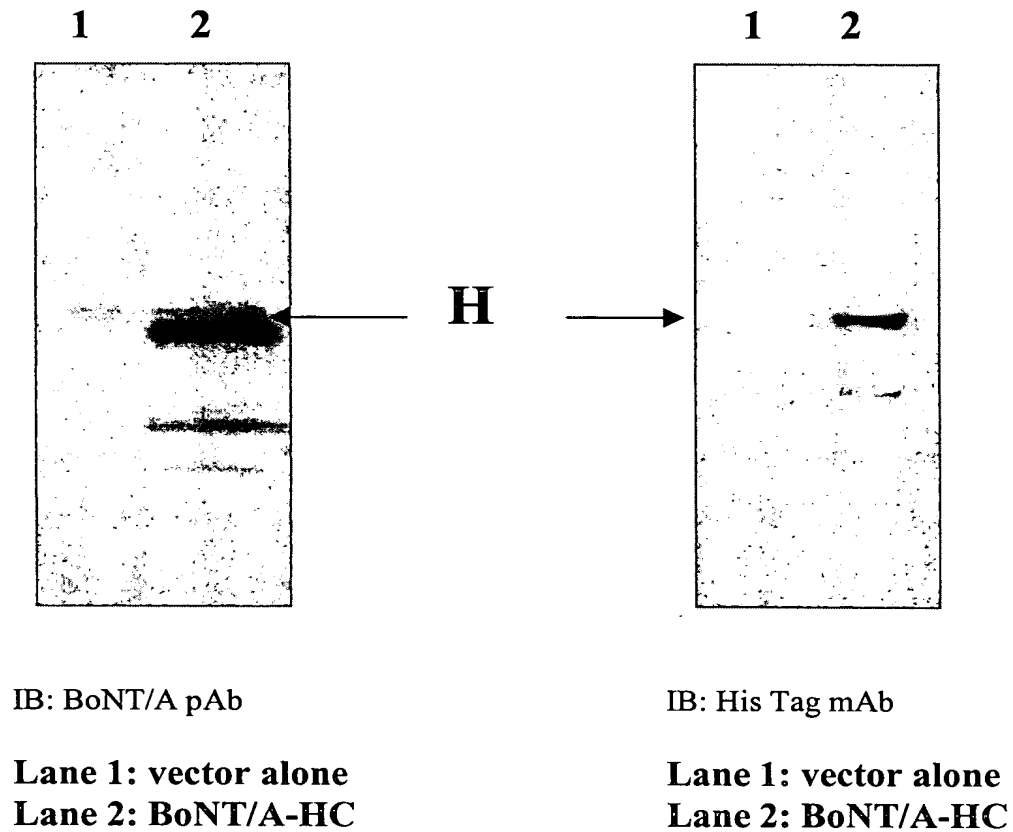


1. pBAC-1/LCA, inactive (H227Y)
2. pBAC-1/LCA, active
3. pBACgus-1/LCA, inactive (H227Y)
4. pBACgus-1/LCA
5. AcNPV, negative control
6. Sf9 insect cell lysate only
7. rLCA, positive control
8. Substrate only

**Figure 6**





**Figure 7.**

**Figure 8.**

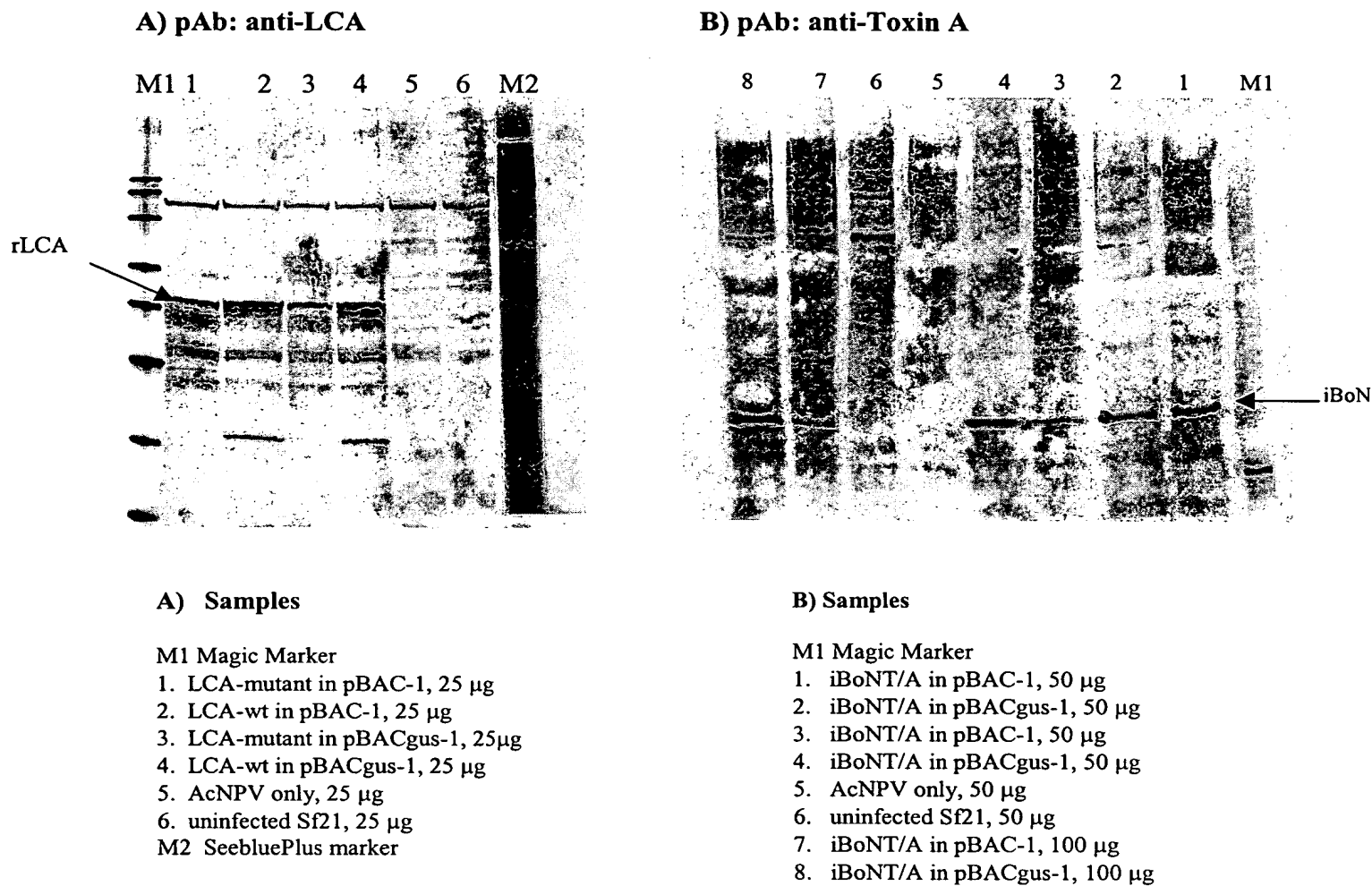
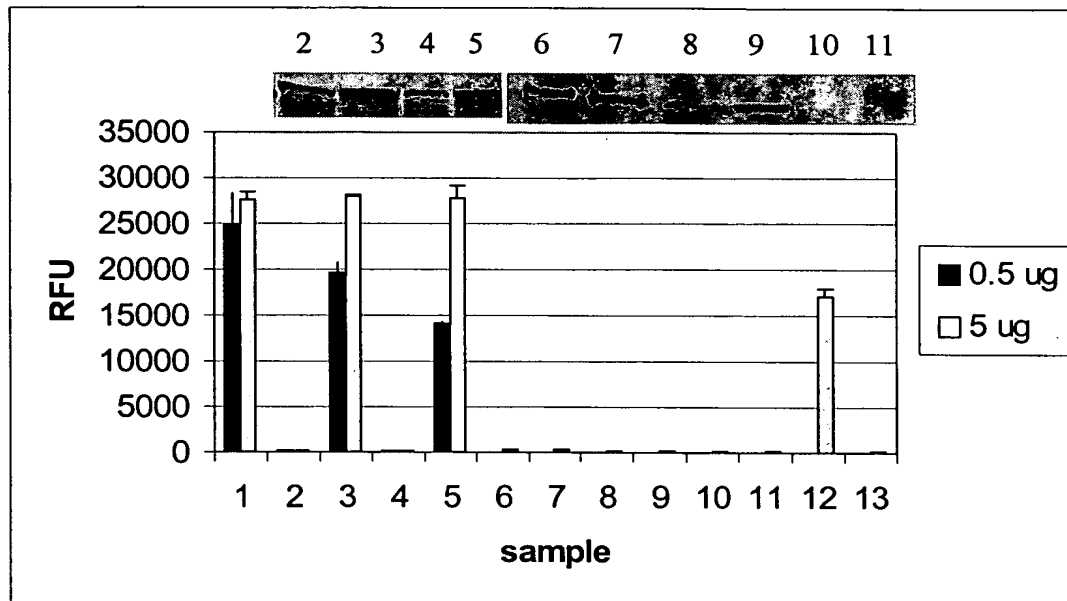


Figure 9.



1. LCA in pBAC-1, 11/02
2. LCA-mutant in pBAC-1
3. LCA-wt in pBAC-1
4. LCA-mutant in pBACgus-1
5. LCA-wt in pBACgus-1
6. iBoNT/A in pBAC-1, 0.5  $\mu$ g
7. iBoNT/A in pBACgus-1, 0.5  $\mu$ g
8. iBoNT/A in pBAC-1, 1  $\mu$ g
9. iBoNT/A in pBACgus-1, 1  $\mu$ g
10. AcNPV only, negative control
11. Uninfected Sf21
12. E.Coli expressed rLCA
13. Substrate

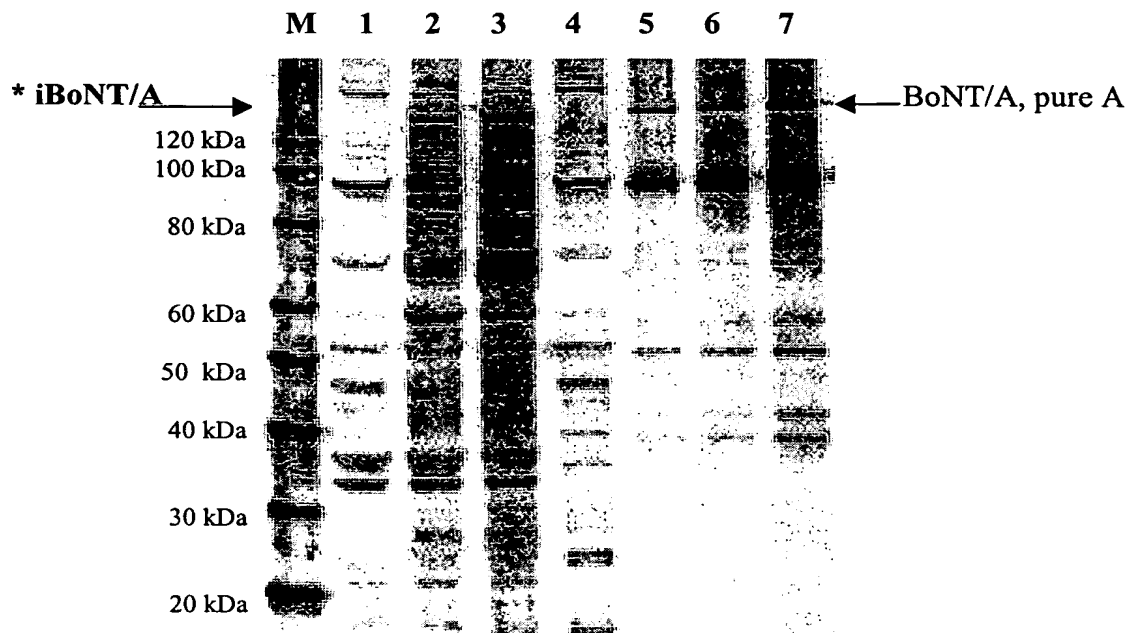
Figure 10

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1      MPFVNKQFNY KDPVNGVDIA YIKIPNAGQM QPVKAFKIHN KIWVIPERDT
51     FTNPEEGDLN PPPEAKQVPV SYDSTYLST DNEKDNYLKG VTKLFERIYS
101    TDLGRMLLTS IVRGIPFWGG STIDTELKVI DTNCINVIQP DGSYRSEELN
151    LVIIGPSADI IQFECKSFGH EVLNLTRNGY GSTQYIRFSP DFTFGFEESL
201    EVDTNPLLGA GKFDTPPAVT LAHELIYAGH RLYGIAINPN RVFKVNTNAY
251    YEMSGLEVSF EELRTFGGHD AKFIDSLQEN EFRLYYYNKF KDIASLTNKA
301    KSIVGTTASL QYMKNVFKEK YLLSEDTSGK FSVDKLKFDK LYKMLTEIYT
351    EDNFVKFFKV LNRKTYLNFD KAVFKINIVP KVNYYTIYDGF NLRNTNLAAN
401    FNGQNTENN MNFTKLKNFT GLFEFYKLLC VRGIITSTTK SLDKGYNKAL
451    NDLCIKVNNW DLFFSPSEDN FTNDLNKGEE ITSDTNIEAA EENISLDLIQ
501    QYLTTFNFDN EPENISIENL SSDIIGQLEL MPNIERFPNG KKYELDKYTM
551    FHYLRAQEFE HGKSRIALTN SVNEALLNPS RVYTFSSDY VKKVNKATEA
601    AMFLGWVEQL VYDFTDETSE VSTTDKIADI TIIIPYIGPA LNIGNMLYKD
651    DFGALIFSG AVILLEFIPE IAIPVLGTFA LVSYIANKVL TVQTIDNALS
701    KRNEKWDEVY KYIVTNWLAK VNTQIDLIRK KMKEALENQA EATKAIINYQ
751    YNQYTEEEKN NINFNIDDL SSKNESINKA MININKFLNQ CSVSYLMNSM
801    IPYGVKRLED FDASLKDALL KYIYDNRGTL IGQVDRLLDK VNNLTSTDIP
851    FQLSKYVDNQ RLLSTFTEYI KNIINTSILN LRYESNHLID LSRVASKINI
901    GSKVNFDPID KNQIQLFNLE SSKIEVILKN AIVYNSMYEN FSTSFWIRIP
951    KYFNSISLNN EYTIINCMEN NSGWKVSLEY GEIIWTLQDT QEIKQRVVFV
1001   YSQMINISDY INRWIFVTIT NNRLNNSKIY INGRLLIDQKP ISNLGNIHAS
1051   NNIMFKLDGC RDTHRYIWIW YFNLFDKELN EKEIKDLYDN QSNSGILKDF
1101   WGDYLYQYDKP YYMLNLYDPN KYVDVNVVGI RGYMYLKGPR GSVMTTNIYL
1151   NSSLYRGTKF IIKKYASGNK DNIVRNNDRV YINVVVKNE YRLATNASQA
1201   GVEKILSALE IPDVGNLSQV VVMKSKNDQG ITNKCKMNLQ DNNGNDIGFI
1251   GFHQFNNAIAK LVASNWNRYQ IERSSRTLGC SWEFIPVDDG WGERPLHHHH
1301   HH

```

Figure 11.



M, MagicMark

1, AcNPV, negative control

2, pBAC-1/iBoNT/A (H227Y)-His6

3, pBACgus-1/iBoNT/A (H227Y)-His6

4, uninfected Sf21 cells

5, Native BoNT/A, Pure A, 10 ng

6, Native BoNT/A, Pure A, 20 ng

7, Native BoNT/A, Pure A, 50 ng